John von Neumann Institute for Computing

Transition Metal Centers in Biological Matrices: Structure and Function of Vanadate in Vanadium Haloperoxidases and as Phosphate Analog

Winfried Plass

published in

NIC Symposium 2001, Proceedings,
Horst Rollnik, Dietrich Wolf (Editor),
John von Neumann Institute for Computing, Jülich,
NIC Series, Vol. **9**, ISBN 3-00-009055-X, pp. 103-110, 2002.

http://www.fz-juelich.de/nic-series/volume9

Transition Metal Centers in Biological Matrices: Structure and Function of Vanadate in Vanadium Haloperoxidases and as Phosphate Analog

Winfried Plass

Fachbereich Chemie und Biologie, Anorganische Chemie II
Universität Siegen, 57068 Siegen, Germany
E-mail: plass@chemie.uni-siegen.de

This research project is intended to elucidate the structure and function of vanadium haloperoxidases as well as the causes and consequences of the chemical analogy between vanadate and phosphate. In this context quantum chemical calculations mostly based on density functional theory are performed on relevant model systems. Along this line an important question is concerned with the role of the protein environment via supramolecular interactions. Although particularly important for the cases considered here, such a supramolecular interplay between metal centers and protein environment is a general feature for metalloenzymes.

1 Introduction

The interest in vanadium chemistry from a biological and pharmacological perspective has exploded over the past 20 years. This is mainly based on the discoveries of the role of vanadium as an insulin mimic, and the presence of vanadium in certain haloperoxidases and nitrogenases[1,2]. A key point for the understanding of how vanadium actually works in biological systems is given by the chemical analogy between vanadates and phosphates. One of the still open questions in this context is whether vanadate generally acts as a stable transition state analog for phosphate blocking specific receptor sites or whether there are cases for which the vanadate substitution actually leads to new active sites with a completely different reactivity with consequences for the pharmacological application and the toxicity of vanadium.

2 Vanadium in Biological Systems

The biological function of vanadium is well established[1,2]. Of particular importance is the ability of vanadium to influence phosphate-metabolizing systems and the fact that vanadium is an inherent part of enzymatic active sites. For both types of vanadoenzymes known today, the vanadium-containing haloperoxidases (see figure 1) and the vanadium nitrogenases from the nitrogen fixing bacteria *Azotobacter*, functional analogs are found in nature which are either more widely spread or more efficient, e.g. the heme-containing haloperoxidases and the conventional nitrogenases with molybdenum cofactor, respectively. This immediately leads to the question of how this enzyme systems did evolve and in particular whether the vanadium-containing enzymes known today are retained functional analogs, which simply could sustain evolutionary forces. New insight concerning these questions may be gained on the basis of newly found similarities for vanadate and phosphate in biological systems[3].

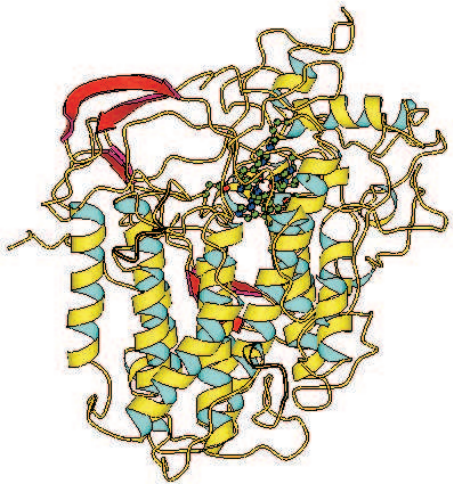

Figure 1. Structure of the chloroperoxidase of the fungus *Curvularia inaequalis* determined by X-ray crystallography.

The wide spread physiological effects of vanadium are mainly attributed to the similarity between its anionic form, vanadate(V), and phosphate. But there are also important differences between these two anions. At physiological pH values monovanadate is found as doubly protonated $[VO_2(OH)_2]^-$ species, whereas phosphate occurs in the monoprotonated form HPO_4^{2-}. This is also important for possible mechanisms of the transport systems for these two anions[4–6]. In addition vanadium is easily reduced under physiological conditions to yield cationic species. The third difference is given by the pronounced ability of vanadium to adopt higher coordination numbers. The higher coordinative flexibility of vanadium can deliberately be used for the structural characterization of phosphate metabolizing enzymes.

3 Phosphate-Vanadate-Analogy

Recently the crystal structures of several stable enzyme aggregates of phosphatases with vanadate as transition state analog have been reported. An interesting example are the protein tyrosine phosphatases[7,8], which are involved in signal transduction mechanisms for controlling and regulating intracellular processes (e.g. the insulin receptor system) — in this context it is worth noting that vanadate complexes show insulin-mimetic effects[9]. In these aggregates the vanadium is trigonal bipyramidal and linked to the protein with a single axial bound cysteine residue, whereas the oxygen atoms of the vanadate moiety are involved in a hydrogen bonding network. A similar structure is found for the active site of rat prostatic acid phosphatase with the complexed transition state analog vanadate[10] (figure 2). In this case the vanadium is linked to the protein through an axial bound histidine residue.

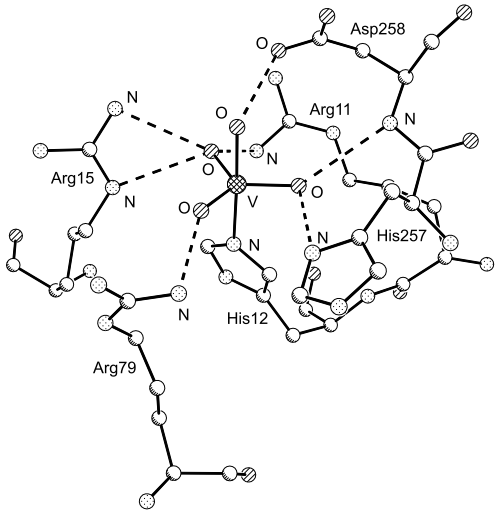

Figure 2. Structure of the active site of the rat prostatic acid phosphatase with complexed vanadate. Hydrogen bonds are shown as broken lines.

Striking similarities are observed for the only structurally characterized vanadoenzymes known today, the vanadium haloperoxidases - e.g. the chloroperoxidase of the fungus *Curvularia inaequalis* (see figure 3)[11–13]. As in the case of the rat prostatic acid phosphatase the vanadate is directly linked to the protein only through the axial bound histidine residue and is embedded in the protein via an extensive hydrogen bonding network.

Vanadium-containing haloperoxidases (V-HPO) are enzymes catalyzing the two electron oxidation of a halide (X^-) to the corresponding hypohalous acid according to Eq. (1)[14].

$$H_2O_2 + X^- + H^+ \longrightarrow H_2O + HOX \qquad (1)$$

HOX may further react with a broad range of nucleophilic acceptors to form a diversity of halogenated compounds. These haloperoxidases are named after the most electronegative halide they are able to oxidize, and thus a vanadium chloroperoxidase (V-CPO) is able to oxidize chloride, bromide and iodide.

Based on the recently published crystal structures of the native and apo forms as well as the peroxide- and azide-bound derivatives of this chloroperoxidase[11–13] several interesting questions still remain and even new once have to be addressed: 1) What is the electronic structure of the active site vanadate moiety, i.e. mono- or dioxovanadium(V) species (VO^{3+} or VO_2^+)? 2) How is the peroxo group or the chloride ion bound to the active site? 3) What is the influence of the protein environment upon the structure of the active site and the mechanism? 4) Does the apo-protein of this chloroperoxidase exhibit

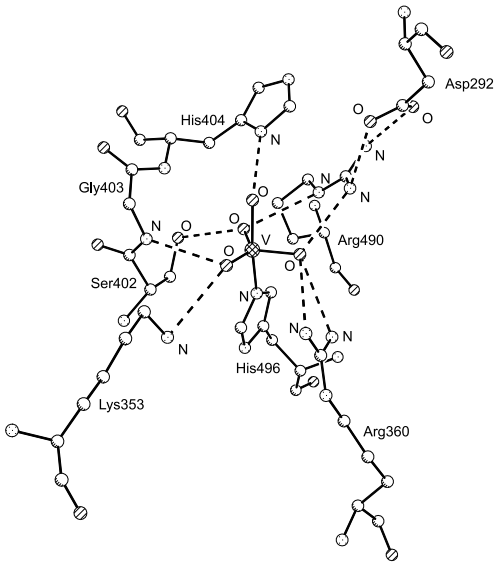

Figure 3. Structure of the active site of vanadium haloperoxidase form the fungus *Curvularia inaequalis*. Hydrogen bonds are shown as broken lines.

phosphatase activity as expected based on its structural similarities to phosphatases?

Concerning the last question an interesting result has recently been published by Wever et al., which were able to show that the apo-protein of the chloroperoxidase isolated from the fungus *Curvularia inaequalis* indeed exhibits phosphatase activity[15]. Although it is obvious from the kinetic data that the active site of this V-CPO is not optimized for phosphatase activity, it is nevertheless clear that within the same supramolecular environment it is possible to catalyse two very different reactions such as those of haloperoxidases and phosphatases. Consequently this structural motive should also be observed for the active sites of other V-HPOs and phosphatases. Moreover, this structural similarity should give rise to genetic relationships as well. According to the sequence alignment of relevant enzymes nearly all amino acid residues coordinating vanadate in V-CPO isolated from *Curvularia inaequalis* (Lys353, Arg360, Ser402, Gly403, His404, Arg490 and His496 see figure 3) are conserved in the V-HPOs sequenced thus far as well as within three families of acid phosphatases (see figure 4)[15]. Within this active site architecture the fully conserved histidine residues play an important role. His496 is the residue covalently linking the vanadate to the protein, whereas His404 is essential for catalysis and is proposed to function as an acid-base group. Together with the observed phosphatase activity of the apo-protein of the V-CPO this indicates a very similar architecture of the active sites of these V-HPOs and acid phosphatases.

The dendrogram depicted in figure 4 is based on the sequence alignment of 14 phosphatases and V-HPOs. These results indicate that these enzymes have divergently evolved from a common ancestor. In combination with the observed stability of V-CPO from

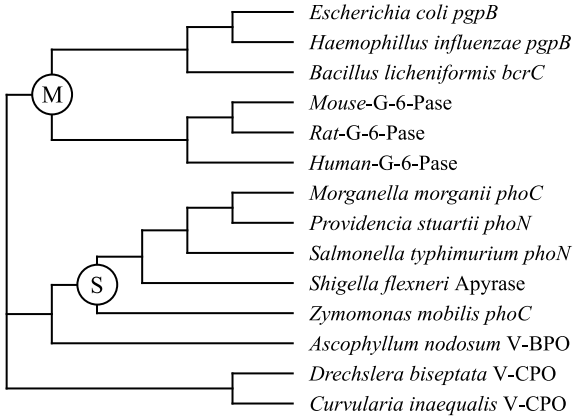

Figure 4. Dendrogram based on the alignment of 14 acid phosphatases and vanadium haloperoxidases. The group of membrane-bound phosphatases is marked with an M, and the group of soluble phosphatases is marked with an S. Abbreviations: *pgpB*, phoshpatdiyl glycerophosphate phosphatase B; *bcrC*, gene product from *Bacillus licheniformis*, no function has been assigned yet; G-6-Pase, glucose-6-phosphatase; *phoC* and *phoN*, class A bacterial acid phosphatase; Apyrase, ATP-diphosphohydrolase; V-BPO, vanadium bromoperoxidase; V-CPO, vanadium chloroperoxidase.

Curvularia inaequalis, which in particular resist a high concentration of their substrate (H_2O_2) and their product (HOX) that would readily inactivate the heme-containing HPOs, this contradicts the introductory hypothesis that V-HPOs are retained enzymatic systems that simply could sustain evolutionary forces. This is further evidenced by the optimized functionality of V-CPO from *Curvularia inaequalis*, that is given by its recently proposed putative role as source for the starting material for the production of a chemical weapon, the hydroxyl radical (see Eq. 2), which in turn can be used by the fungus to damage the protective lignocellulose of plant tissues allowing the penetration of the plant cell for nutrient acquisition[16].

$$\mathrm{HOCl} + (\mathrm{O_2})^{\bullet -} \longrightarrow (\mathrm{OH})^{\bullet} + \mathrm{Cl}^{-} + \mathrm{O_2} \qquad (2)$$

It is remarkable, however, that for another class of acid phosphatases, that is exemplified by the rat prostatic acid phosphatase (see figure 2), no sequence similarity is found with the enzymes given in the dendrogram of figure 4. Nevertheless the observed structural similarity of the active site, obvious from figures 2 and 3, indicates that a convergent evolution seems to have occurred with respect to these enzyme systems.

Besides the interesting evolutionary aspects of these enzymes, there are also important implications for both research in the acid phosphatase and in the V-HPO field. A first remarkable example for this — in particular from an anthropocentric point of view — is the structure of mammalian glucose-6-phosphatase (G-6-Pase). This enzyme catalyzes the last step in both gluconeogenesis and glycogenolysis and as such it is the key enzyme in glucose homeostasis. G-6-Pase deficiency is the cause of glycogen storage disease

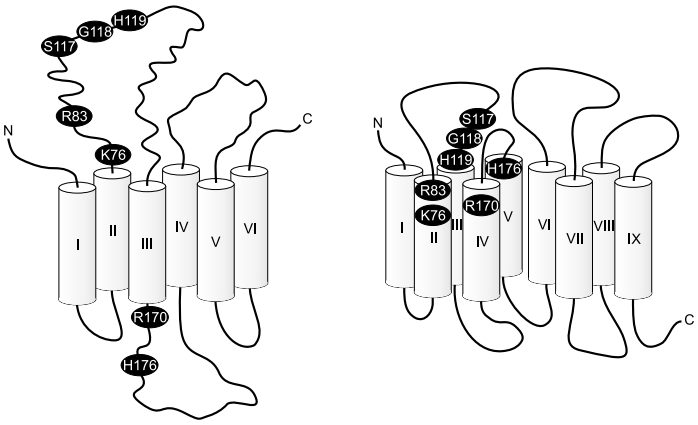

Figure 5. Membrane topology models for G-6-Pase. Left: Current six transmembrane-helix topology model. Right: Newly proposed nine transmembrane-helix topology model. Putative G-6-Pase active site residues are depicted as closed ovals.

type 1 (von Gierke disease) and is characterized by severe clinical manifestations such as hypoglycemia, kidney enlargement or growth retardation, which usually cause an early death of the patients.

The recently published model for the membrane topology of human G-6-Pase[17] is not consistent with the results outlined above and had to be modified accordingly as depicted in figure 5[18]. The active site residues of G-6-Pase identified by sequence alignment are all situated on the same side of the membrane and are located within the helices II, III, IV and V. In this new model the histidine residue His176 is the nucleophile forming the phosphohistidine enzyme-substrate intermediate. The phosphate moiety is positioned by interaction of the negatively charged oxygens with the positively charged amino acids Lys76, Arg83 and Arg170. In analogy to the active site of V-CPO the residues Ser117 and Gly118 may also donate hydrogen bonds and the histidine group His119 may provide the proton needed to liberate the glucose moiety.

As shown by this first example, the common architecture of the active sites of the vanadium-containing peroxidases and the aligned acid phosphatases has important implications, that even reach into seemingly independent fields of research.

4 Mechanism of Vanadium Haloperoxidases

The current understanding of the catalytic activity of V-HPOs based on chemical, biochemical and crystallographic data is summarized in figure 6. It raises new questions. What controls the specific turnover activity, which can vary over 1000-fold depending on the source of the enzyme? What defines the basis of the halide specificity in V-BPO and V-CPO?

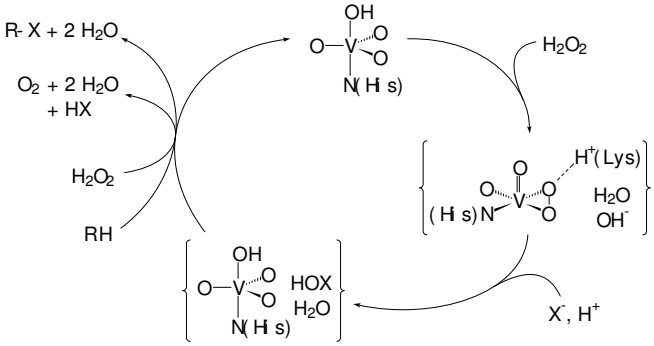

Figure 6. Schematic representation of the current description of the catalytic activity of V-HPOs for the two possible reactions ($RH + H_2O_2 + HX \rightarrow RX + 2\ H_2O$ and $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$.

To answer these questions a more detailed knowledge on the molecular mechanism of the reactions at the active site is needed. In particular information on possible reaction intermediates is not available at present. Therefore theoretical investigations on suitable model systems including the supramolecular environment of the protein matrix are part of the present project.

This leads to an additional point of interest related to the influence of the protein environment on the particular reactivity of the active site. This is particularly important since the apo-protein of the chloroperoxidase isolated from the fungus *Curvularia inaequalis* exhibits phosphatase activity[15]. This clearly indicates that within the same supramolecular environment it is possible to catalyse two very different reactions such as those of haloperoxidases and phosphatases. A proposed mechanism for the phosphatase

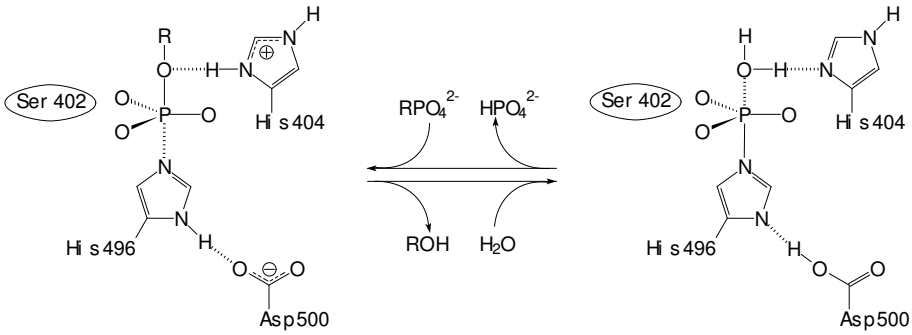

Figure 7. Schematic representation of the suggested mechanism for the catalytic activity of membrane bound phosphatases (numbering scheme of the amino acids is related to the V-CPO structure (see figure 3).

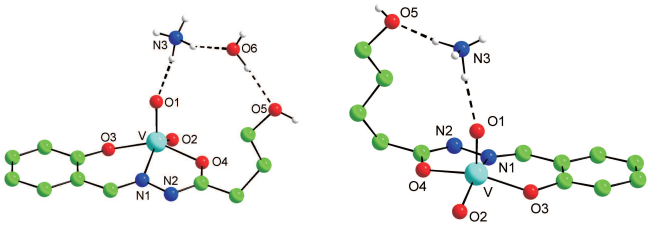

Figure 8. Structures of model systems for the supramolecular interaction of vanadate species with relevant protein matrices.

activity of the V-HPO protein environment is depicted in figure 7.

5 Molecular Model Systems

For a detailed understanding of the reactivity of the biological systems described above a comparison with chemical models is important. This is in particular the case when it comes to the question of the influence of the protein environment on the reactivity. We have synthesized a series of such model systems which exhibit hydrogen bonding at the vanadate center related to the observed supramolecular environment of the native V-HPO proteins (see figures 8)[19,20]. The hydrogen bonding network for one of the model systems as observed in the crystal structure is depicted in figure 9.

These model systems in addition also are used to calibrate the methodology of our theoretical investigations. This is important, since the description of hydrogen bonds within theoretical models is a difficult task.

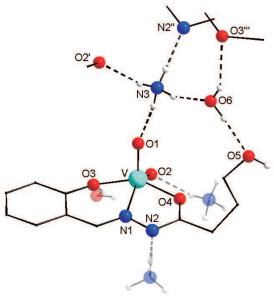

Figure 9. Representation of the hydrogen bonding network for one of the model compounds depicted in figure 8 which exhibits features similar to the supramolecular environment of the active site structure of V-HPOs (see figure 3).

6 Computational Methods

The theoretical investigations related to this project are based on density functional (DFT) calculations. The choice of both the appropriate density functional and the employed basis sets is verified by calculations on model systems ranging from simple vanadate moieties to molecular assemblies like the once depicted in figure 8. The mechanistic aspects for the protein related systems are addressed by QM/MM methods in which the major part of the protein environment is treated on a molecular mechanics (MM) basis and the key part of the active site by quantum mechanics (QM). For medium sized systems representing the active site structure including the important part of the supramolecular protein environment also molecular dynamics simulations based on the Car-Parrinello method are part of the project. All calculations have been performed at the computer center (ZAM) of the Forschungszentrum Jülich utilizing two massively-parallel computers (CRAY T3E-600 and CRAY T3E-1200) and a vector-parallel computer (CRAY T90). The computer system used for a particular application depends on the efficiency of the implemented software and the size of the molecular assembly to be calculated. For the QM/MM calculations and the molecular dynamics simulations the CRAY massively-parallel computers turned out to be essential tools with an average job size of 128 nodes with about 2 hours of CPU time.

Acknowledgments

This project is supported by a grant of computer time of the John von Neumann Institute for Computing (NIC) and a research grant of the Deutsche Forschungsgemeinschaft.

References

1. C. Slebodnick, B. J. Hamstra, V. L. Pecoraro, *Struct. Bonding* **1997**, *89*, 51–108.
2. *Metal Ions in Biological Systems, Vol. 31: Vanadium and Its Role in Life* (Eds.: H. Sigel, A. Sigel), Marcel Dekker, New York, **1995**.
3. W. Plass, *Angew. Chemie. Int. Ed.* **1999**, *38*, 909–912.
4. F. A. Quiocho, P. S. Ledvina, *Mol. Microbiol.* **1996**, *20*, 17–25.
5. H. Luecke, F. A. Quiocho, *Nature* **1990**, *347*, 402–406.
6. O. Herzberg, P. Reddy, S. Sutrina, J. M. H. Saier, J. Reizer, G. Kapadia, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 2499–2503.
7. J. M. Denu, D. L. Lohse, J. Vijayalakshmi, M. A. Saper, J. E. Dixon, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 2493–2498.
8. M. Zhang, M. Zhou, R. L. van Etten, C. V. Stauffacher, *Biochemistry* **1997**, *36*, 15–23.
9. C. Orvig, K. H. Thompson, M. Battell, J. H. McNeill in *Metal Ions in Biological Systems, Vol. 31: Vanadium and Its Role in Life* (Eds.: H. Sigel, A. Sigel), Marcel Dekker, New York, **1995**, pp. 575–594.
10. Y. Lindqvist, G. Schneider, P. Vihko, *Eur. J. Biochem.* **1994**, *221*, 139–142.
11. A. Messerschmidt, R. Wever, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 392–396.

12. A. Messerschmidt, L. Prade, R. Wever, *Biol. Chem.* **1997**, *378*, 309–315.
13. A. Messerschmidt, R. Wever, *Inorg. Chim. Acta* **1998**, *273*, 160–166.
14. S. L. Neidleman, J. Geigert, *Biohalogenation: Principles, Basic Roles and Applications*, Ellis Horwood, Chichester, **1986**.
15. W. Hemrika, R. Renirie, H. L. Dekker, P. Barnett, R. Wever, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2145–2149.
16. P. Barnett, D. L. Kruitbosch, W. Hemrika, H. L. Dekker, R. Wever, *Biochim. Biophys. Acta* **1997**, *1352*, 73–84.
17. K.-J. Lei, C.-J. Pan, J.-L. Liu, L. L. Shelly, J. Y. Chou, *J. Biol. Chem.* **1995**, *270*, 11882–11886.
18. W. Hemrika, R. Wever, *FEBS Lett.* **1997**, *409*, 317–319.
19. W. Plass, A. Pohlmann, H.-P. Yozgatli, *J. Inorg. Biochem.* **2000**, *80*, 181–183.
20. W. Plass, A. Pohlmann, *J. Inorg. Biochem.* **2001**, *86*, 381.